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(54) Multi-specimen slides for immunohistologic procedures.

(57) A process for producing a slide bearing a spaced array of specimen fragments which comprises (i) cutting at least one specimen into a plurality of narrow strips; (ii) separating the plurality into groups of specimen strips; (iii) separately positioning strips from the groups in parallel grooves in a mold; (iv) embedding the strips in the mold in a first embedding medium to provide a structure comprising a base member having opposed first and second surfaces, the first surface being substantially planar; the second surface having ridges containing a specimen strip extending therefrom; (v) forming a stack of the structures with the terminal surface of the ridges of an upper structure abutting the substantially planar first surface of the next lower structure; the spaces between the ridges defining channels for receipt of a fluid; (vi) embedding the stack in a second embedding medium to form a block having a spaced array of parallel specimen strips embedded therein; the strips being so arranged that a section of the block includes a spaced array of cross-sections of each of the embedded specimen strips; (vii) dividing the block into sections each containing a spaced array of cross-sections of each of the embedded specimen strips; (viii) mounting at least one of such block sections on a slide.

EP 0 350 189 A2

MULTI-SPECIMEN SLIDES FOR IMMUNOHISTOLOGIC PROCEDURES

BACKGROUND OF THE INVENTION

This invention relates to multi-specimen slides useful in immunohistologic procedures. More particularly, the invention relates to slides bearing a plurality of specimens in spaced array appropriate for automated image analysis and to technology germane to such slides.

Various multi-specimen slides are known. Paraffin block sections each containing multiple tissue specimens are described in Lillie, Histopathologic Technic and Practical Histochemistry, McGraw-Hill, Inc., New York, New York (1965) pp. 74-77. Composite snap-frozen tissue sections mounted on a slide for use in diagnostic autoimmunology are described in Nairn, Fluorescent Protein Tracing, 4th Ed., Churchill Livingstone, London (1976) pp. 131-138. Johnson, et al. Handbook of Experimental Immunology, 3rd Ed., Blackwell Scientific Publications, Oxford, England (1978) refers to composite frozen tissues useful for autoantibody testing with the admonition that "To get satisfactory sections the tissue pieces must be frozen together without leaving spaces between them..." (p. 154). Mason, et al. in Bullock, et al. Techniques in Immunocytochemistry, Vol. 2, Academic Press, London (1983) pp. 175-216 states that tissue culture supernatants may be tested again either paraffin embedded sections or cryostat sections of snap-frozen tissue. Cryostat sections may be placed in the wells of multitest slides (pp. 192-193). Mason also states that hybridoma supernatants may be tested on air dried cell smears (p. 192). Battifora describes a multitissue tumor block useful for immunohistochemical antibody testing in Laboratory Investigation 55:244-248 (1986). Various multitissue slides are described in Stocker U.S. patent 4,647,543.

Computer controlled automatic image analysis instruments useful with appropriate software to analyze the spaced specimen array of slides of this invention are commercially available. Typical instruments include Recognition Concepts, Inc, Gould DeAnza, Inc. and Megabesion, Inc.

SUMMARY OF THE INVENTION

This invention provides slides bearing a plurality of specimen fragments in spaced array appropriate for automated computer-controlled image analysis. The specimen fragments may be of any kind. Fixed or frozen unfixed tissue specimens and cell culture specimens are preferred. The invention also subsumes technology germane to the production and use of such slides.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a slide in accordance with the invention.

Figure 2 is a perspective view of a multiblade device for cutting specimens into strips.

Figure 3 is a perspective view of a mold provided with parallel grooves to receive specimen strips.

Figure 4 is a perspective view of an embedding medium structure having specimen strips containing ridges of a type formed from the mold of Figure 3.

Figure 5 is a perspective view of a stack of structures as shown in Figure 4.

Figure 6 is a perspective view of a container having perforated walls for receiving a stack of structures as depicted by Figure 5.

Figure 7 is a perspective view of a section as produced by a microtome or the like of a block as depicted in Figure 6.

DETAILED DESCRIPTION OF THE INVENTION

Slides pursuant to the invention bear a plurality of specimen fragments in a spaced array. The pattern of the array may be selected to accommodate computer controlled image analysis. Quadrangular, i.e., square or rectangular patterns are preferred.

The invention is particularly concerned with slides useful in immunohistologic procedures. Such slides typically have tissues or cell culture specimen fragments mounted thereon. Either fixed or unfixed, frozen tissue specimens may be used. For many purposes, frozen tissue slides are preferred to insure the preservation of substantially unmodified tissue components such as antigens. The tissue specimens may be stained in known manner.

Figure 1 illustrates a slide 10 bearing a plurality of tissue specimen fragments 11 in a substantially equally spaced rectangular array. In practice, the spacing may be arranged to accommodate automated image analysis. For example, a minimum of 3 pixels or about 75 to 100 microns space between specimens at a magnification of 25 times with a 512 x 512 array is appropriate.

Slides in accordance with the invention are

appropriately provided with fragments from a plurality of different relatively large tissue or cell culture specimens. Each relatively large specimen is cut into narrow strips in any appropriate manner, for example, with a multiblade cutting device as illustrated by Figure 2. Referring to the figure, the device comprises a series of blades 12 separated by spacer means 13 of an appropriate dimension to provide specimen strips of a desired narrow width. The cutting device knives and spacers are mounted on support means 14, each of which includes a removable retention means 15.

The relatively large tissue specimens for subdivision into narrow strips may be obtained from any available source such as autopsies or operations. Cell culture samples may, for example, be suspended in a gel, and the gel poured over a plate and dried to provide a layer of appropriate thickness, preferably about 0.5 to about 1.5 mm, and the layer thereafter removed from the plate and cut into narrow strips with a device as shown in Figure 2. Cell culture smears formed in known manner, see Mason, supra at page 193, comprise another source of specimen strips.

Strips 16 of fixed or of unfixed frozen tissue or of cell culture are placed in the parallel grooves 17 of a mold such as the mold 18 illustrated by Figure 3. An appropriate embedding medium, e.g., agar gel, is added to the mold containing the specimen strips and allowed to solidify thus producing a solidified embedding medium structure 19 as illustrated by Figure 4 upon removal from the mold 18.

The structure 19 comprises embedding medium in the form of a base member 20 having a substantially planar surface 21 and an opposed surface 22 having a plurality of spaced ridges 23 extending therefrom. Each ridge 23 includes a specimen strip 16.

A plurality of structures 19 are stacked as shown by Figure 5. In the stack, the terminal surfaces 24 of each ridge 23 abut the planar surface of the adjacent lower structure. The spaces between ridges provide channels 25 for access of fluids such as fixatives to the specimen strips in the ridges.

The stack of structures is placed in a container 26 as shown by Figure 6. The container walls include perforations 27 to permit the ingress and egress of fluids such as clearing and dehydrating agents.

A fixative may be introduced into and passed through the channels 25 to condition the specimen strips for further processing.

After fixing, the stack of structures 19 is removed from the container 26 and placed in a deep mold for final embedding to form a multispecimen block. The final embedding medium may be conventional, for example, paraffin or another wax, a high molecular weight polyethylene glycol or polyvinyl alcohol, nitrocellulose, a methacrylate resin, or an epoxy resin.

The block is sectioned by a microtome or like device to provide a plurality of sections 28, each containing a spaced array of specimen sections as shown in Figure 7. In the spaced array the channels 25 are filled by the final embedding material.

The block sections are mounted in known manner to provide slides of the kind indicated generally by the slide 10 of Figure 1.

To produce slides of the invention bearing fragments of unfixed frozen tissue or of frozen cell cultures, snap-frozen unfixed, preferably different, specimens are cut into narrow strips, placed while frozen in the parallel grooves 18 of a mold such as the mold 18, and embedded in an embedding medium such as OCT appropriate for use in freeze drying procedures to produce frozen structures 19 of the kind illustrated by Figure 4. Such structures, while frozen are stacked and the stack is embedded in a final embedding medium to provide a frozen block containing a plurality of spaced, parallel specimen strips as shown generally by Figure 7. The block is sectioned, e.g., by a cryostat to provide sections containing a plurality of specimen fragments in spaced array also as shown by Figure 7. The sections are mounted, in known manner, while frozen on slides and may thereafter be freeze dried.

Specimen fragments on the slides of this invention may be arranged in defined segments in which related specimen fragments are grouped together or associated in a manner to facilitate automated image processing. For example, one run of specimens, each of different, but known characteristics, may be positioned across a slide, e.g., a top run, to provide standards. Columns of unknown specimens may be provided above or below each standard included.

Claims

1. A process for producing a slide bearing a spaced array of specimen fragments which comprises:

(i) cutting at least one specimen into a plurality of narrow strips;

(ii) separating said plurality into groups of specimen strips;

(iii) separately positioning strips from said groups in parallel grooves in a mold;

(iv) embedding said strips in said mold in a first embedding medium to provide a structure comprising a base member having opposed first and second surfaces, said first surface being substantially planar;

said second surface having ridges containing a specimen strip extending therefrom;

(v) forming a stack of said structures with the terminal surface of said ridges of an upper structure abutting the substantially planar first surface of the next lower structure;
the spaces between said ridges defining channels for receipt of a fluid;

(vi) embedding said stack in a second embedding medium to form a block having a spaced array of parallel specimen strips embedded therein; said strips being so arranged that a section of said block includes a spaced array of cross-sections of each of said embedded specimen strips;

(vii) dividing said block into sections each containing a spaced array of cross-sections of each of said embedded specimen strips;

2. The process of claim 1 in which the specimen comprises a fixed tissue, a frozen unfixed tissue. or a cell culture.

3. The process of claim 1 in which

(i) the specimen is a tissue fixed for storage;

(ii) said first embedding medium is agar gel or gelatin;

(iii) the stack of structures formed in step (v) is placed in contact with a fixative which occupies the channels defined by the spaces between the ridges of said structures;

(iv) said second embedding medium is paraffin, polyethylene glycol, a methacrylate resin or an epoxy resin.

4. A process for producing a slide bearing a spaced array of unfixed frozen or freeze dried tissue specimens which comprises

(i) cutting unfixed, frozen tissue specimens into a plurality of narrow strips;

(ii) separating said plurality into groups of frozen strips;

(iii) separately positioning the strips from each of said groups in parallel grooves in a mold;

(iv) embedding said so positioned strips in said mold in a cryogenic embedding medium to provide a frozen structure comprising a base member having opposed first and second surfaces
said first surface being substantially planar;
said second surface having a plurality of ridges containing a specimen strip extending therefrom;

(v) forming a stack of said frozen structures with the terminal surface of said ridges of an upper stack abutting the planar surface of the next lower structure;

(vi) embedding the frozen stack in a cryogenic embedding medium to produce a frozen embedding medium block having a spaced array of parallel specimen strips embedded therein
said strips being so arranged that a section of said block includes a spaced array of cross-sections of each of said specimen strips;

(vii) dividing said frozen block into sections each containing a spaced array of frozen cross-sections of each of said strips;

(viii) mounting at least one of said frozen sections on a slide.

5. A slide bearing specimen fragments in a spaced array appropriate for automated image analysis.

6. A slide as bearing frozen, unfixed tissue fragments in a spaced array appropriate for automatic image analysis.

7. A slide as defined by claim 6 on which the tissue fragments are freeze dried.

8. A slide bearing fixed tissue fragments in a spaced array appropriate for automatic image analysis.

9. A structure comprising
a base member formed from an embedding medium;
said base member having opposed first and second surfaces
said first surface being substantially planar;
said second surface having a plurality of spaced parallel ridges extending therefrom; and
specimen strips in at least some of said ridges.

10. A structure as defined in claim 9 in which said specimen strips are strips of fixed tissue, frozen unfixed tissue or of a cell culture composition.

11. A structure as defined by claim 9 in which said specimen strips are strips of fixed tissue and the embedding medium is agar gel or gelatin.

12. A structure as defined by claim 9 in which said specimen strips are strips of frozen, unfixed tissue and the embedding medium is cryogenic.

13. A structure as defined by claim 9 or claim 10 in which said ridges have substantially planar terminal surfaces.

14. A stack of structures as defined by claim 9 or claim 10 in which
the terminal surfaces of the ridges or an upper structure in said stack abut the substantially planar first surface of the next lower stack;
the spaces between said ridges defining parallel channels.

15. A process for substantially simultaneously fixing a plurality of tissue specimens which comprises introducing a fixative into the channels in a stack of structures as defined by claim 14 to contact the tissue specimens present in the ridges of the structures comprising said stack.

16. An embedding medium block having a spaced array of specimen strips embedded therein, said strips being so arranged that a section of said block normal to the longitudinal axis of said strips includes a spaced array of cross-sections of each of said embedded strips.

17. A block as defined by claim 16 in which specimen strips comprise fixed tissue, unfixed frozen tissue or a cell culture composition.

18. A multispecimen slide comprising a row of different specimens of known characteristics and a plurality of unknown specimens positioned above or below at least one of the known specimens in said row to provide at least a column including one known specimen and a plurality of unknown specimens.

19. A multispecimen slide as defined in claim 18 in which said known and unknown specimens are specimens of a cell culture composition or a fixed tissue.

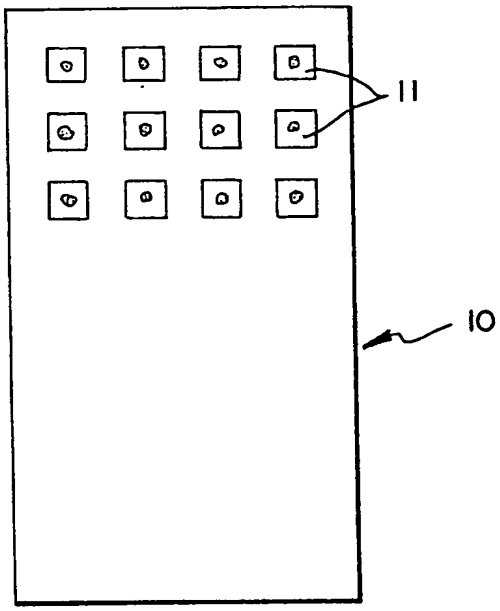


FIG. 1

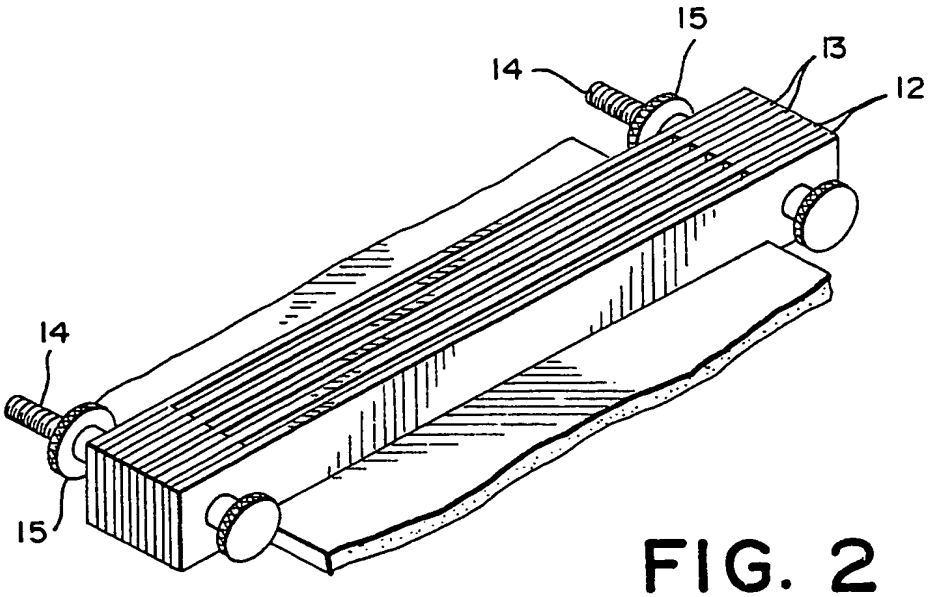


FIG. 2

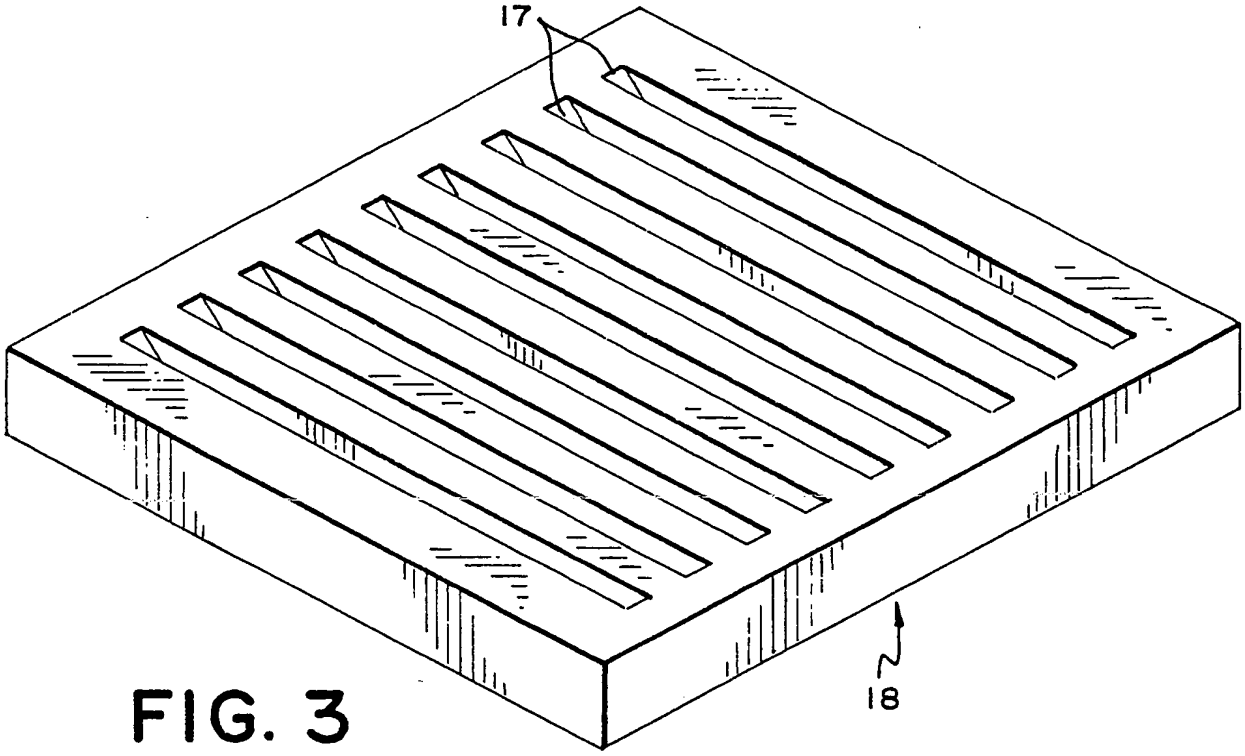


FIG. 3

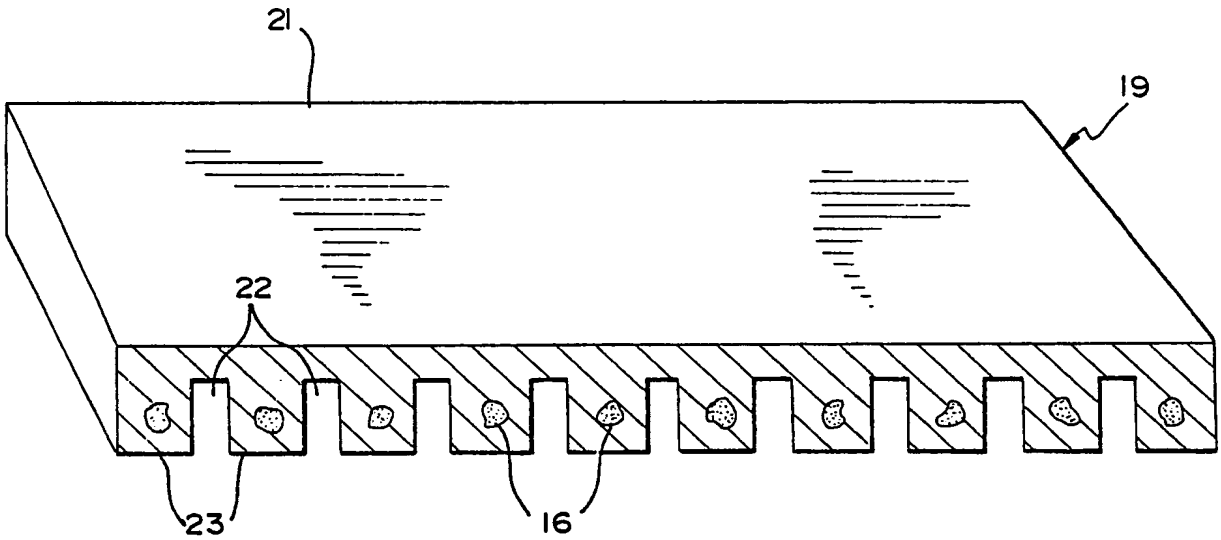


FIG. 4

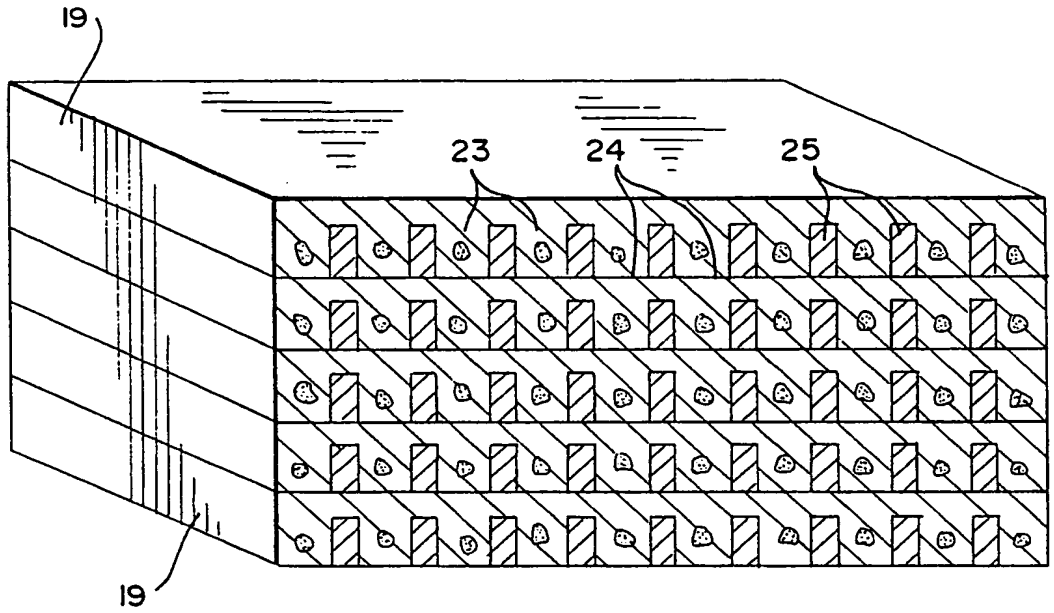


FIG. 5

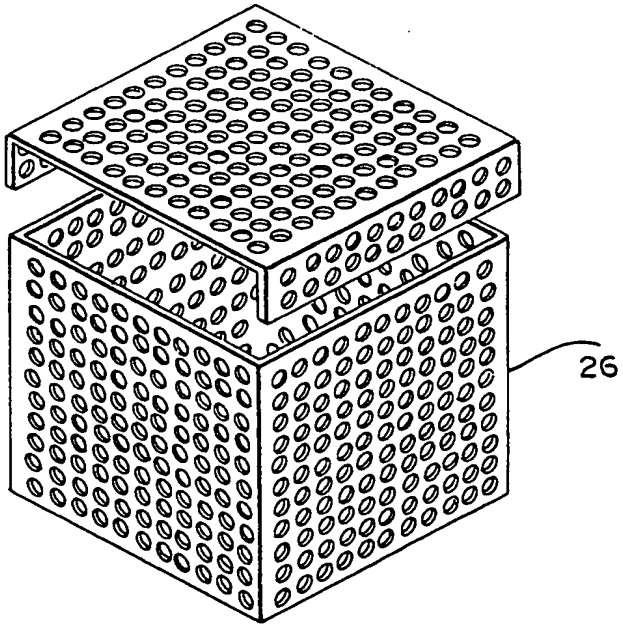
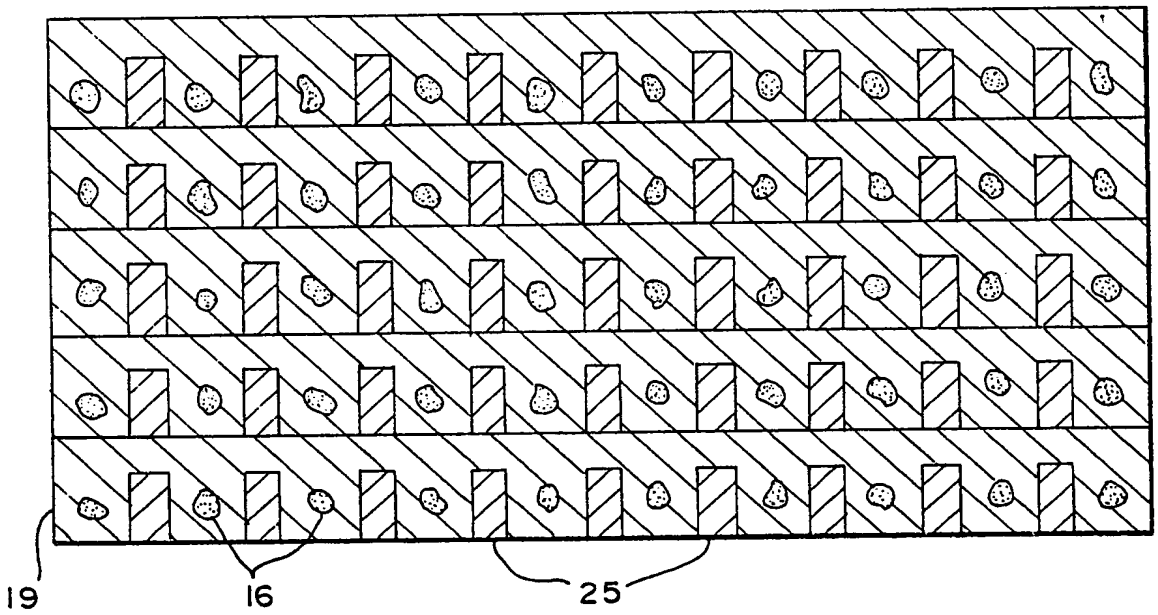


FIG. 6

FIG. 7



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(54) Multi-specimen slides for immunohistologic procedures.

(57) A process for producing a slide bearing a spaced array of specimen fragments which comprises (i) cutting at least one specimen into a plurality of narrow strips; (ii) separating the plurality into groups of specimen strips; (iii) separately positioning strips from the groups in parallel grooves in a mold; (iv) embedding the strips in the mold in a first embedding medium to provide a structure comprising a base member having opposed first and second surfaces, the first surface being substantially planar; the second surface having ridges containing a specimen strip extending therefrom; (v) forming a stack of the structures with the terminal surface of the ridges of an upper structure abutting the substantially planar first surface of the next lower structure; the spaces between the ridges defining channels for receipt of a fluid; (vi) embedding the stack in a second embedding medium to form a block having a spaced array of parallel specimen strips embedded therein; the strips being so arranged that a section of the block includes a spaced array of cross-sections of each of the embedded specimen strips; (vii) dividing the block into sections each containing a spaced array of cross-sections of each of the embedded specimen strips; (viii) mounting at least one of such block sections on a slide.

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 30 6458

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 238 190 (BECKMAN RESEARCH INSTITUTE OF THE CITY OF HOPE) * Column 3, line 23 - column 4, line 35; column 5, lines 22-48 *	1	G 01 N 1/28
A,D	US-A-4 647 543 (W. STÖCKER)		

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

G 01 N

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
THE HAGUE	25-02-1991	HOCQUET A.P.E.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

European Patent Office

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

X LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,
namely:

See sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,

namely claims: 1-4,9-17

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

1. Claims 1-4,9-17: Process for producing a slide and structures obtained by this process.

2. Claims 5-8: Slide adapted for automatic image analysis.

3. Claims 18-19: Slide with two different portions for known and unknown specimens.

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(12) # EUROPEAN PATENT SPECIFICATION

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(56) References cited:
EP-A- 0 238 190
US-A- 4 647 543

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EP 0 350 189 B1

Description

BACKGROUND OF THE INVENTION

This invention relates to multi-specimen slides useful in immunohistologic procedures. More particularly, the invention relates to slides bearing a plurality of specimens in spaced array appropriate for automated image analysis and to technology germane to such slides.

Various multi-specimen slides are known. Paraffin block sections each containing multiple tissue specimens are described in Lillie, Histopathologic Technic and Practical Histochemistry, McGraw-Hill, Inc., New York, New York (1965) pp. 74-77. Composite snap-frozen tissue sections mounted on a slide for use in diagnostic autoimmunology are described in Nairn, Fluorescent Protein Tracing, 4th Ed., Churchill Livingstone, London (1976) pp. 131-138. Johnson, et al. Handbook of Experimental Immunology, 3rd Ed., Blackwell Scientific Publications, Oxford, England (1978) refers to composite frozen tissues useful for autoantibody testing with the admonition that "To get satisfactory sections the tissue pieces must be frozen together without leaving spaces between them..." (p. 154). Mason, et al. in Bullock, et al. Techniques in Immunocytochemistry, Vol. 2, Academic Press, London (1983) pp. 175-216 states that tissue culture supernatants may be tested again either paraffin embedded sections or cryostat sections of snap-frozen tissue. Cryostat sections may be placed in the wells of multitest slides (pp. 192-193). Mason also states that hybridoma supernatants may be tested on air dried cell smears (p. 192). Battifora describes a multitissue tumor block useful for immunohistochemical antibody testing in Laboratory Investigation 55:244-248 (1986). Various multitissue slides are described in Stocker U.S. patent 4,647,543. EP-A-0 238 190 discloses a process for producing a slide bearing an irregularly arranged array of specimen fragments.

Computer controlled automatic image analysis instruments useful with appropriate software to analyze the spaced specimen array of slides of this invention are commercially available. Typical instruments include Recognition Concepts, Inc, Gould DeAnza, Inc. and Megabesion, Inc.

SUMMARY OF THE INVENTION

This invention provides slides bearing a plurality of specimen fragments in regularly spaced array appropriate for automated computer-controlled image analysis. The specimen fragments may be of any kind. Fixed or frozen unfixed tissue specimens and cell culture specimens are preferred. The invention also subsumes technology germane to the production and use of such slides.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a slide in accordance with the invention.

Figure 2 is a perspective view of a multiblade device for cutting specimens into strips.

Figure 3 is a perspective view of a mold provided with parallel grooves to receive specimen strips.

Figure 4 is a perspective view of an embedding medium structure having specimen strips containing ridges of a type formed from the mold of Figure 3.

Figure 5 is a perspective view of a stack of structures as shown in Figure 4.

Figure 6 is a perspective view of a container having perforated walls for receiving a stack of structures as depicted by Figure 5.

Figure 7 is a perspective view of a section as produced by a microtome or the like of a block as depicted in Figure 6.

DETAILED DESCRIPTION OF THE INVENTION

Slides pursuant to the invention bear a plurality of specimen fragments in a spaced array. The pattern of the array may be selected to accommodate computer controlled image analysis. Quadrangular, i.e., square or rectangular patterns are preferred.

The invention is particularly concerned with slides useful in immunohistologic procedures. Such slides typically have tissues or cell culture specimen fragments mounted thereon. Either fixed or unfixed, frozen tissue specimens may be used. For many purposes, frozen tissue slides are preferred to insure the preservation of substantially unmodified tissue components such as antigens. The tissue specimens may be stained in known manner.

Figure 1 illustrates a slide 10 bearing a plurality of tissue specimen fragments 11 in a substantially equally spaced rectangular array. In practice, the spacing may be arranged to accommodate automated image analysis. For example, a minimum of 3 pixels or about 75 to 100 μm space between specimens at a magnification of 25 times with a 512 x 512 array is appropriate.

Slides in accordance with the invention are appropriately provided with fragments from a plurality of different relatively large tissue or cell culture specimens. Each relatively large specimen is cut into narrow strips in any appropriate manner, for example, with a multiblade cutting device as illustrated by Figure 2. Referring to the figure, the device comprises a series of blades 12 separated by spacer means 13 of an appropriate dimension to provide specimen strips of a desired narrow width. The cutting device knives and spacers are mounted on support means 14, each of which includes a removable retention means 15.

The relatively large tissue specimens for subdivision into narrow strips may be obtained from any

available source such as autopsies or operations. Cell culture samples may, for example, be suspended in a gel, and the gel poured over a plate and dried to provide a layer of appropriate thickness, preferably about 0.5 to about 1.5 mm, and the layer thereafter removed from the plate and cut into narrow strips with a device as shown in Figure 2. Cell culture smears formed in known manner, see Mason, supra at page 193, comprise another source of specimen strips.

Strips 16 of fixed or of unfixed frozen tissue or of cell culture are placed in the parallel grooves 17 of a mold such as the mold 18 illustrated by Figure 3. An appropriate embedding medium, e.g., agar gel, is added to the mold containing the specimen strips and allowed to solidify thus producing a solidified embedding medium structure 19 as illustrated by Figure 4 upon removal from the mold 18.

The structure 19 comprises embedding medium in the form of a base member 20 having a substantially planar surface 21 and an opposed surface 22 having a plurality of spaced ridges 23 extending therefrom. Each ridge 23 includes a specimen strip 16.

A plurality of structures 19 are stacked as shown by Figure 5. In the stack, the terminal surfaces 24 of each ridge 23 abut the planar surface of the adjacent lower structure. The spaces between ridges provide channels 25 for access of fluids such as fixatives to the specimen strips in the ridges.

The stack of structures is placed in a container 26 as shown by Figure 6. The container walls include perforations 27 to permit the ingress and egress of fluids such as clearing and dehydrating agents.

A fixative may be introduced into and passed through the channels 25 to condition the specimen strips for further processing.

After fixing, the stack of structures 19 is removed from the container 26 and placed in a deep mold for final embedding to form a multispecimen block. The final embedding medium may be conventional, for example, paraffin or another wax, a high molecular weight polyethylene glycol or polyvinyl alcohol, nitrocellulose, a methacrylate resin, or an epoxy resin.

The block is sectioned by a microtome or like device to provide a plurality of sections 28, each containing a spaced array of specimen sections as shown in Figure 7. In the spaced array the channels 25 are filled by the final embedding material.

The block sections are mounted in known manner to provide slides of the kind indicated generally by the slide 10 of Figure 1.

To produce slides of the invention bearing fragments of unfixed frozen tissue or of frozen cell cultures, snap-frozen unfixed, preferably different, specimens are cut into narrow strips, placed while frozen in the parallel grooves 17 of a mold such as the mold 18, and embedded in an embedding medium appropriate for use in freeze drying procedures to produce frozen structures 19 of the kind illustrated by Figure 4. Such structures, while frozen are stacked and the stack is embedded in a final embedding medium to provide a frozen block containing a plurality of spaced, parallel specimen strips as shown generally by Figure 7. The block is sectioned, e.g., by a cryostat to provide sections containing a plurality of specimen fragments in spaced array also as shown by Figure 7. The sections are mounted, in known manner, while frozen on slides and may thereafter be freeze dried.

Specimen fragments on the slides of this invention may be arranged in defined segments in which related specimen fragments are grouped together or associated in a manner to facilitate automated image processing. For example, one run of specimens, each of different, but known characteristics, may be positioned across a slide, e.g., a top run, to provide standards. Columns of unknown specimens may be provided above or below each standard included.

Claims

1. A process for producing a slide (10) bearing an array of specimen fragments (11) comprising

cutting at least one specimen into a plurality of narrow strips; and

separating said plurality into groups of specimen strips (16); characterized in that the array is a regularly spaced array and the process further comprises

(i) separately positioning strips (16) from said groups in parallel grooves (17) in a mold (18);
(ii) embedding said strips (16) in said mold (18) in a first embedding medium to provide a structure (19) comprising a base member (20) having opposed first and second surfaces,

said first surface (21) being substantially planar;

said second surface having ridges (23) containing a specimen strip (16) extending therefrom;

(iii) forming a stack of said structures with the terminal surface (24) of said ridges (23) of an upper structures abutting the substantially planar first surface (21) of the next lower structure;

the spaces between said ridges defining channels (25) for receipt of a fluid;

(iv) embedding said stack in a second embedding medium to form a block having a spaced array of parallel specimen strips (16) embedded therein;

said strips (16) being so arranged that a section (28) of said block includes a spaced array of cross-sections of each of said embedded specimen strips;

(v) dividing said block into sections each containing a spaced array of cross-sections of

each of said embedded specimen strips;
(vi) mounting at least one of such block sections on a slide (10).

2. The process of claim 1 in which the specimen comprises a fixed tissue, a frozen unfixed tissue, or a cell culture.

3. The process of claim 1 in which
(i) the specimen is a tissue fixed for storage;
(ii) said first embedding medium is agar gel or gelatin;
(iii the stack of structures formed in step (iii) is placed in contact with a fixative which occupies the channels (25) defined by the spaces between the ridges (23) of said structures;
(iv) said second embedding medium is paraffin, polyethylene glycol, a methacryiate resin or an epoxy resin.

4. A process according to claim 1 wherein the said specimen is of unfixed frozen or freeze dried tissue wherein
in step (ii) the first embedding medium is a cryogenic embedding medium and said structure is a frozen structure;
in step (iii) the said structures are frozen structures;
in step (iv) the said stack is a frozen stack and the said second embedding medium is cryogenic embedding medium
in step (v) the said block and cross-sections are frozen
in step (vi) the said sections are frozen.

5. A slide (10) bearing specimen fragments in a regularly spaced array appropriate for automated image analysis obtainable by the process of any one of claims 1 to 4.

6. A structure for use in a process according to any of claims 1-4 comprising
a base member formed from an embedding medium;
said base member (20) having opposed first and second surfaces
said first surface (21) being substantially planar;
said second surface having a plurality of spaced parallel ridges (23) extending therefrom; and
specimen strips (16) in at least some of said ridges.

7. A structure as defined in claim 6 in which said specimen strips (16) are strips of fixed tissue, frozen unfixed tissue or of a cell culture composition.

8. A structure as defined by claim 6 in which said specimen strips (16) are strips of fixed tissue and the embedding medium is agar gel or gelatin.

9. A structure as defined by claim 6 in which said specimen strips (16) are strips of frozen, unfixed tissue and the embedding medium is cryogenic.

10. A structure as defined by claim 6 or claim 7 in which said ridges (23) have substantially planar terminal surfaces.

11. A stack of structures as defined by claim 6 or claim 7 in which
the terminal surfaces (24) of the ridges (23) or an upper structure in said stack abut the substantially planar first surface (21) of the next lower stack;
the spaces between said ridges defining parallel channels (25).

12. A process for substantially simultaneously fixing a plurality of tissue specimens which comprises introducing a fixative into the channels in a stack of structures as defined by claim 11 to contact the tissue specimens present in the ridges of the structures comprising said stack.

13. An embedding medium block for use in a process according to any of claims 1-4 having an array of specimen strips (16) embedded therein, said strips being so arranged that a section (28) of said block normal to the longitudinal axis of said strips includes an array of cross-sections of each of said embedded strips characterized in that the array is a regularly spaced array.

14. A block as defined by claim 13 in which specimen strips (16) comprise fixed tissue, unfixed frozen tissue or a cell culture composition.

15. A slide (10) according to claim 5 comprising a row of different specimens of known characteristics and a plurality of unknown specimens positioned above or below at least one of the known specimens in said row to provide at least a column including one known specimen and a plurality of unknown specimens.

16. A slide (10) as defined in claim 15 in which said known and unknown specimens are specimens of a cell culture composition or a fixed tissue.

Patentansprüche

1. Ein Verfahren zur Herstellung eines eine Anordnung bzw. Reihe von Untersuchungsprobenfragmente (11) tragenden Objektträgers (10), umfassend

Schneiden mindestens einer Untersuchungsprobe in eine Anzahl von schmalen Streifen und

Trennen der Anzahl in Gruppen von Untersuchungsprobestreifen (16),

dadurch gekennzeichnet, daß die Anordnung bzw. Reihe eine gleichmäßig beabstandete Anordnung ist und das Verfahren weiterhin umfaßt

(i) getrenntes Positionieren von Streifen (16) der Gruppen in parallele Nuten bzw. Vertiefungen (17) in einer Form (18),

(ii) Einbetten der Streifen (16) in der Form (18) in einem ersten Einbettungsmedium zum Schaffen einer Struktur (19) mit einem Grundelement (20) mit gegenüberliegenden ersten und zweiten Oberflächen, wobei die erste Oberfläche (21) im wesentlichen planar ist und die zweite Oberfläche von dieser abstehende, einen Untersuchungsprobestreifen (16) enthaltende Rippen bzw. Kanten (23) aufweist,

(iii) Bilden eines Stapels der Strukturen, bei dem die Endfläche (24) der Rippen bzw. Kanten (23) einer oberen Struktur in Anlage mit der im wesentlichen planaren ersten Oberfläche (21) der nächsten unteren Struktur ist, wobei die Zwischenräume zwischen den Kanten bzw. Rippen Kanäle (25) zur Aufnahme eines Fluids festlegen,

(iv) Einbetten des Stapels in ein zweites Einbettungsmedium zum Bilden eines Blocks mit einer beabstandeten Anordnung paralleler in diesem eingebetteter Untersuchungsprobestreifen (16), wobei die Streifen (16) so angeordnet sind, daß ein Abschnitt (28) des Blocks eine beabstandete Anordnung von Querschnitten jedes der eingebetteten Untersuchungsprobestreifen umfaßt,

(v) Unterteilen des Blocks in Abschnitte, von denen jeder eine beabstandete Anordnung von Querschnitten jedes der eingebetteten Untersuchungsprobestreifen enthält,

(vi) Befestigen mindestens eines der derartigen Blockabschnitte auf einem Objektträger (10).

2. Das Verfahren gemäß Anspruch 1, wobei die Untersuchungsprobe ein fixiertes Gewebe, ein gefrorenes unfixiertes Gewebe oder eine Zellenkultur umfaßt.

3. Das Verfahren gemäß Anspruch 1, wobei

(i) die Untersuchungsprobe ein zur Lagerung fixiertes Gewebe ist,

(ii) das erste Einbettungsmedium ein Agargel oder Gelatine ist,

(iii) der Stapel von im Schritt (iii) gebildeten Strukturen in Berührung mit einem Fixiermittel angeordnet wird, welches sich in den durch die Zwischenräume zwischen den Rippen bzw. Kanten (23) der Struktur festgelegten Kanälen (25) befindet,

(iv) das zweite Einbettungsmedium Paraffin, Polyethylenglycol, ein Methacrylatharz oder ein Epoxyharz ist.

4. Ein Verfahren gemäß Anspruch 1, wobei die Untersuchungsprobe ein unfixiertes gefrorenes oder gefriergetrocknetes Gewebe umfaßt, wobei

im Schritt (ii) das erste Einbettungsmedium ein niedere Temperaturen erzeugendes Einbettungsmedium und die Struktur eine gefrorene Struktur ist,

im Schritt (iii) die Strukturen gefrorene Strukturen sind,

im Schritt (iv) der Stapel ein gefrorener Stapel und das zweite Einbettungsmedium ein niedrige Temperaturen erzeugendes Einbettungsmedium ist,

im Schritt (v) der Block und die Querschnitte gefroren sind,

im Schritt (vi) die Abschnitte gefroren sind.

5. Ein Objektträger (10), der Untersuchungsprobenfragmente in einer regelmäßig beabstandeten Anordnung trägt, die für eine automatisierte Bildanalyse geeignet ist und die bzw. der durch das Verfahren gemäß einem der Ansprüche 1 bis 4 herstellbar ist.

6. Eine Struktur zur Verwendung in einem Verfahren gemäß einem der Ansprüche 1 bis 4, umfassend

ein aus einem Einbettungsmedium gebildetes Grundelement, wobei das Grundelement (20) gegenüberliegende erste und zweite Oberflächen aufweist, wobei die erste Oberfläche (21) im wesentlichen planar ist und die zweite Oberfläche eine Anzahl beabstandeter paralleler Rippen bzw. Kanten (23), die von dieser abstehen, sowie Untersuchungsprobenstreifen (16) in wenigstens einigen der Kanten bzw. Rippen aufweist.

7. Eine Struktur gemäß Anspruch 6, wobei die Untersuchungsprobenstreifen (16) Streifen eines fixierten Gewebes, eines gefrorenen unfixierten Gewebes oder eines Zellenkulturgemisches sind.

8. Eine Struktur gemäß Anspruch 6, wobei die Untersuchungsprobenstreifen (16) Streifen eines fixierten Gewebes sind und das Einbettungsmedium Agargel oder Gelatine ist.

9. Eine Struktur gemäß Anspruch 6, wobei die Untersuchungsprobenstreifen (16) Streifen eines gefrorenen, unfixierten Gewebes sind und das Einbettungsmedium ein niedere Temperaturen erzeugendes Medium ist.

10. Eine Struktur gemäß Anspruch 6 oder 7, wobei die Rippen bzw. Kanten (23) im wesentlichen planare Endflächen aufweisen.

11. Ein Stapel von gemäß Anspruch 6 oder 7 definierten Strukturen, wobei

die Endflächen (24) der Rippen bzw. Kanten (23) oder eine obere Struktur in dem Stapel an der im wesentlichen planaren ersten Oberfläche (21) des nächsten unteren Stapels anliegen und

die Zwischenräume zwischen den Rippen bzw. Kanten parallele Kanäle (25) festlegen.

12. Ein Verfahren zum im wesentlichen gleichzeitigen Fixieren einer Anzahl von Gewebe-Untersuchungsproben, welches ein Einführen eines Fixiermittels in die Kanäle in einem Stapel gemäß Anspruch 11 definierter Strukturen, um die in den Rippen bzw. Kanten der den Stapel umfassenden Strukturen vorliegenden Gewebe-Untersuchungsproben damit in Berührung zu bringen.

13. Ein Einbettungsmediumblock zur Verwendung in einem Verfahren gemäß einem der Ansprüche 1 bis 4 mit einer Anordnung von in diesem eingebetteten Untersuchungsprobenstreifen (16), wobei die Streifen so angeordnet sind, daß ein Abschnitt (28) des Blockes, der senkrecht zu der Längsachse der Streifen ist, eine Anordnung von Querschnitten jedes der eingebetteten Streifen umfaßt, dadurch gekennzeichnet, daß die Anordnung eine regelmäßig beabstandete Anordnung ist.

14. Ein Block gemäß Anspruch 13, in dem die Untersuchungsprobenstreifen (16) ein fixiertes Gewebe, ein unfixiertes, gefrorenes Gewebe oder ein Zellenkulturgemisch umfassen.

15. Ein Objektträger (10) gemäß Anspruch 5, umfassend eine Reihe unterschiedlicher Untersuchungsproben bekannter Eigenschaften und eine Anzahl unbekannter Untersuchungsproben, die über oder unter mindestens einer der bekannten Untersuchungsproben in der Reihe angeordnet sind, um mindestens eine Spalte bzw. Säule mit einer bekannten Untersuchungsprobe und einer Anzahl unbekannter Untersuchungsproben zu bilden.

16. Ein Objektträger (10) gemäß Anspruch 15, wobei die bekannten und unbekannten Untersuchungsproben Untersuchungsproben eines Zellkulturgemisches oder eines fixierten Gewebes sind.

Revendications

1. Un procédé pour la production d'un porte-objet (10) portant une disposition de fragments (11) d'échantillons, consistant à :

découper au moins un échantillon en une pluralité de tranches étroites ; et

séparer cette pluralité en groupes de tranches d'échantillon (16) ;

caractérisé en ce que la disposition est une disposition espacée régulièrement, et le procédé consiste également à

(i) positionner séparément les tranches (16) provenant de ces groupes dans les gorges parallèles (19) dans un moule (18)

(ii) inclure ces tranches (16) dans le moule (18) dans un premier milieu d'inclusion pour donner une structure (19) comprenant un élément de base (20) ayant une première et une seconde surfaces opposées,

ladite première surface (21) étant pratiquement plane ;

ladite seconde surface ayant des crêtes (23) qui en partent et contiennent une tranche d'échantillon (16) ;

(iii) former une pile de ces structures, la surface terminale (24) des crêtes (23) d'une structure supérieure venant buter contre la première surface (21) pratiquement plane de la structure inférieure suivante ;

les espaces entre les crêtes définissant des canaux (25) pour recevoir un liquide ;

(iv) inclure cette pile dans un second milieu d'inclusion pour former un bloc dans lequel est incluse une disposition espacée de tranches d'échantillon parallèles (16) ;

ces tranches (16) étant disposées de telle sorte qu'une coupe (28) de ce bloc comprend une disposition espacée de coupes transversales de chacune de ces tranches d'échantillon incluses ;

(v) diviser ledit bloc en coupes dont chacune contient une disposition espacée de coupes transversdales de chacune de ces tranches d'échantillon incluses ;

(vi) monter au moins une de ces coupes de bloc sur un porte-objet (10).

2. Le procédé de la revendication 1 dans lequel l'échantillon comprend un tissu fixé, un tissu non fixé en congélation, ou une culture cellulaire.

3. Le procédé de la revendication 1 dans lequel
(i) l'échantillon est un tissu fixé en vue de stockage ;
(ii) le premier milieu d'inclusion est du gel d'agarose ou de la gélatine ;
(iii) la pile de structures formée au cours de l'étape (iii) est mise en contact avec un fixateur qui occupe les canaux (25) définis par les espaces entre les crêtes (23) de ces structures ;
(iv) le second milieu d'inclusion est de la paraffine, du polyéthylène glycol, une résine méthacrylate ou une résine époxy.

4. Un procédé selon la revendication 1 dans lequel l'échantillon vient d'un tissu non fixé congelé ou lyophilisé et dans lequel :
dans l'étape (ii) le premier milieu d'inclusion est un milieu d'inclusion cryogénique et la structure est une structure en congélation ;
dans l'étape (iii) la structure est une structure en congélation ;
dans l'étape (iv) la pile est une pile en congélation et le second milieu d'inclusion est un milieu d'inclusion cryogénique ;
dans l'étape (v) le bloc et les coupes transversales sont en congélation ;
dans l'étape (vi) les coupes sont en congélation.

5. Un porte-objet (10) portant des fragments d'échantillon dans une disposition espacée régulièrement appropriée à l'analyse d'images automatisée, qui peut être obtenu par le procédé de l'une ou l'autre des revendications 1 à 4.

6. Une structure à utiliser dans un procédé selon l'une ou l'autre des revendications 1 à 4 comprenant
un élément de base formé dans un milieu d'inclusion;
cet élément de base (20) ayant une première et une seconde surfaces opposées
ladite première surface (21) étant pratiquement plane ;
ladite seconde surface ayant une pluralité de crêtes parallèles espacées (23) qui en partent ; et
des tranches d'échantillon (16) dans au moins certaines des crêtes.

7. Une structure telle que définie par la revendication 6 dans laquelle les tranches d'échantillon (16) sont des tranches de tissu fixé, de tissu non fixé en congélation ou d'une composition de culture cellulaire.

8. Une structure telle que définie par la revendication 6 dans laquelle les tranches d'échantillon (16) sont des tranches de tissu fixé et le milieu d'inclusion est du gel d'agarose ou de la gélatine.

9. Une structure telle que définie par la revendication 6 dans laquelle les tranches d'échantillon (16) sont des tranches de tissu non fixé en congélation et le milieu d'inclusion est cryogénique.

10. Une structure telle que définie par la revendication 6 ou la revendication 7 dans laquelle les crêtes (23) ont des surfaces terminales pratiquement planes.

11. Une pile de structures telle que définie par la revendication 6 ou la revendication 7 dans laquelle
les surfaces terminales (24) des crêtes (23) ou une structure supérieure de ladite pile viennent buter contre la première surface pratiquement plane (21) de la pile inférieure suivante ;
les espaces entre les crêtes définissent des canaux parallèles (25).

12. Un procédé pour fixer pratiquement simultanément une pluralité d'échantillons de tissu, qui consiste à introduire un fixateur dans les canaux d'une pile de structures telle que définie par la revendication 11 pour qu'il vienne au contact des échantillons de tissu présents dans les crêtes des structures constituant ladite pile.

13. Un bloc de milieu d'inclusion à utiliser dans un procédé selon l'une ou l'autre des revendications 1 à 4 dans lequel est incluse une disposition de tranches d'échantillon (16), ces tranches étant disposées de telle sorte qu'une coupe (28) de ce bloc perpendiculaire à l'axe longitudinal de ces tranches comprend une disposition de coupes transversales de chacune des tranches ainsi incluses, caractérisé en ce que la disposition est régulièrement espacée.

14. Un bloc tel que défini par la revendication 13 dans lequel les tranches d'échantillon (16) comprennent un tissu fixé, un tissu non fixé en congélation ou une composition de culture cellulaire.

15. Un porte-objet (10) selon la revendication 5 comprenant une rangée d'échantillons différents ayant des caractéristiques connues et une pluralité d'échantillons inconnus placés au-dessus ou au-dessous d'au moins un des échantillons connus de cette rangée pour constituer au moins

une colonne comprenant un échantillon connu et une pluralité d'échantillons inconnus.

16. Un porte-objet (10) tel que défini dans la revendication 15 dans lequel lesdits échantillons connus et inconnus sont des échantillons d'une composition de culture cellulaire ou d'un tissu fixé.

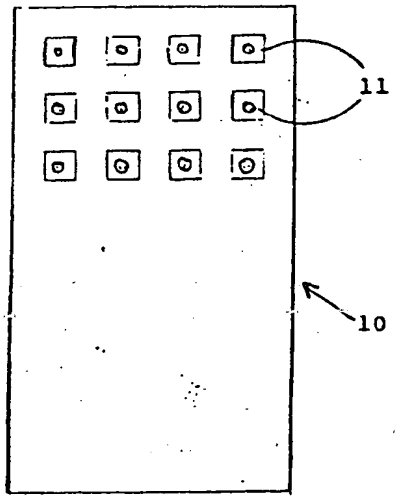


FIGURE 1

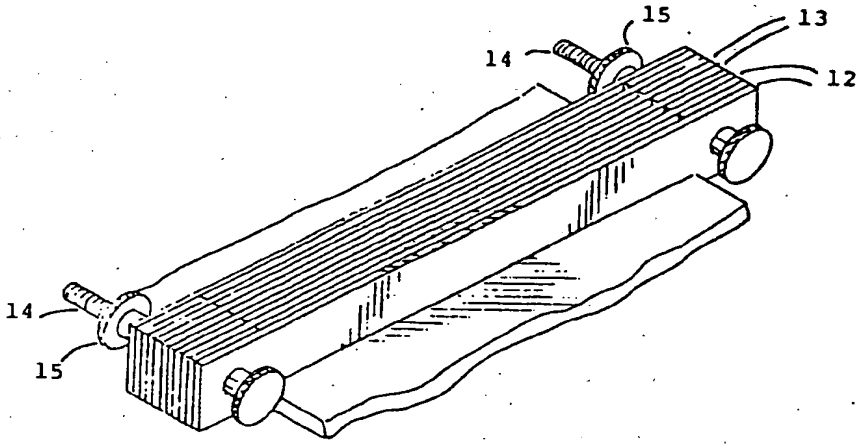


FIGURE 2

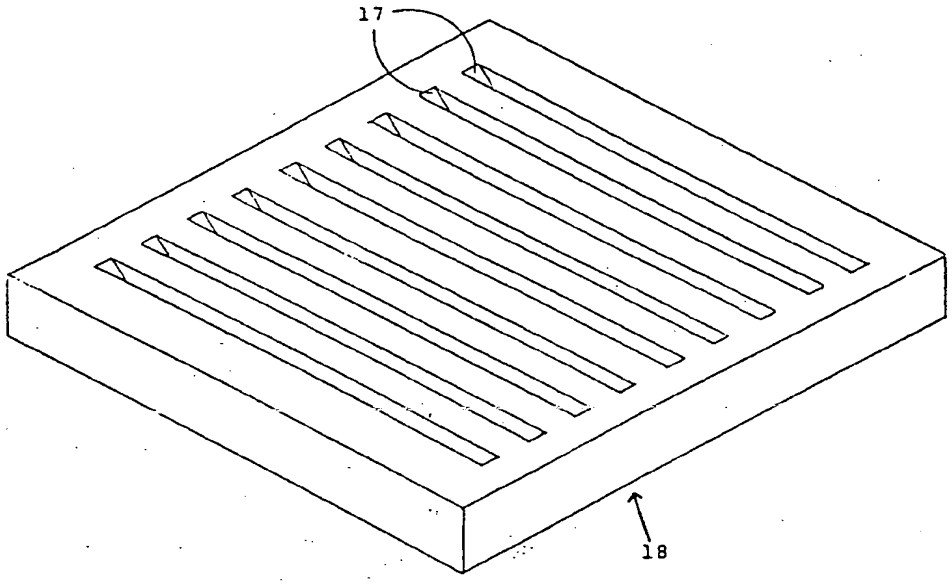


FIGURE 3

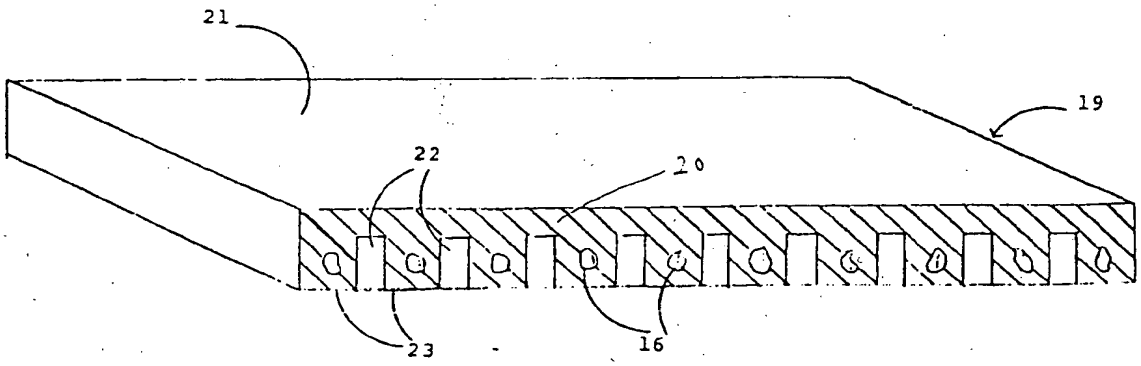


FIGURE 4

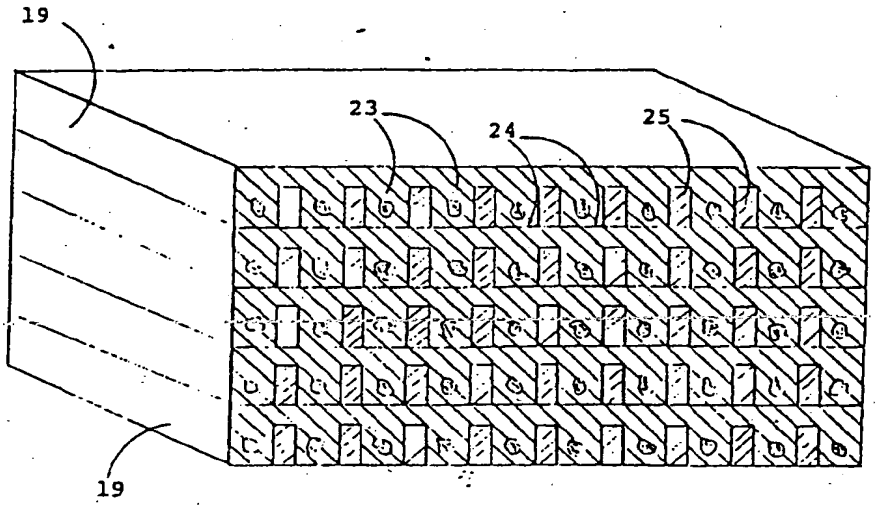
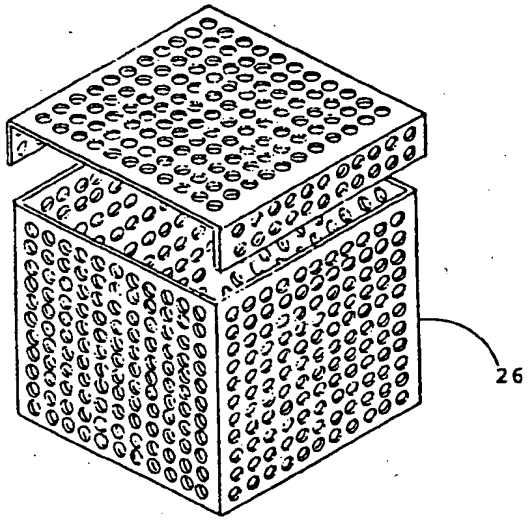


FIGURE 5

FIGURE 6



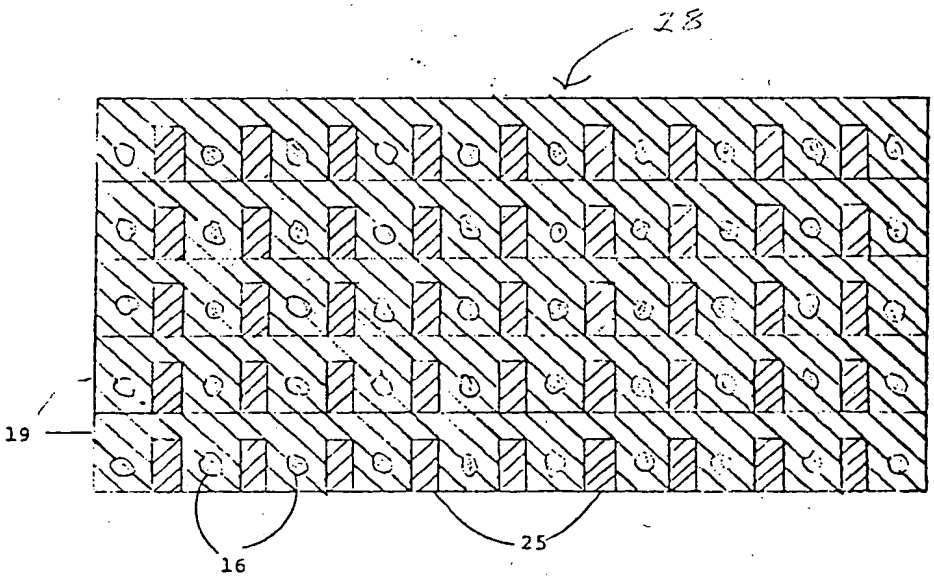


FIGURE 7